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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 21/64 — A2

(11) International Publication Number: WO 00/58715

(43) International Publication Date: 5 October 2000 (05.10.00)

(21) International Application Number: PCT/GB00/01050

(22) International Filing Date: 27 March 2000 (27.03.00)

(30) Priority Data:
9906929.6 26 March 1999 (26.03.99) GB

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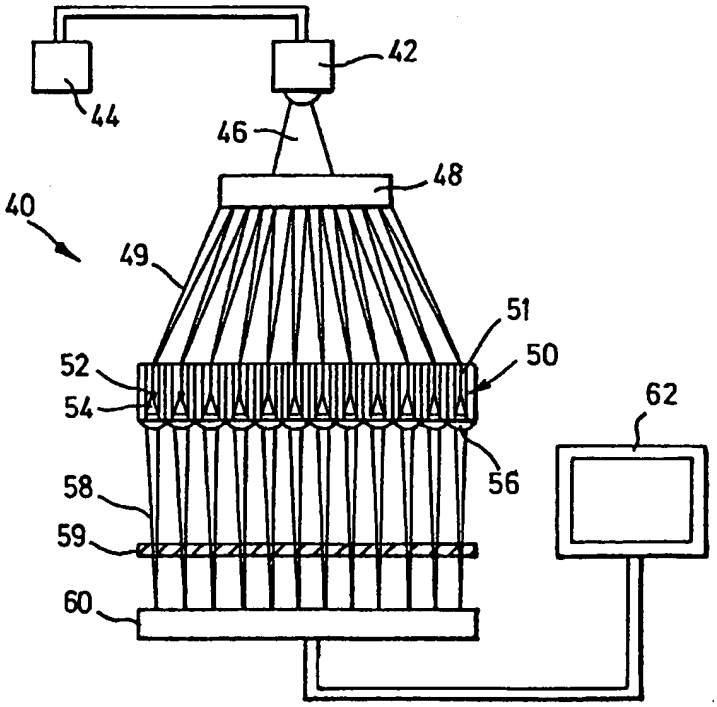
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ASSAY SYSTEM

(57) Abstract

A method and apparatus are disclosed for performing multiple biochemical assays simultaneously. The apparatus (40) includes a laser light source (42), a diffraction grating (48) to diffract the light into multiple excitation beams, a sample holder (50) containing multiple samples (52) to be assayed, and a detection device (60) for detecting radiation either emitted or transmitted by the samples. The invention has application in fluorescence assays, or absorbence assays such as ELISAs. Also disclosed are sample holders for use in the method described, and diffraction gratings. A further modification describes the use of the method for generating DNA chips and the like by photochemistry.



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ASSAY SYSTEM

The present invention relates to apparatus and to a method for use in assaying samples particularly, but not exclusively, for use in high throughput screening of biomolecules.

Modern molecular biology and drug discovery techniques often involve assaying large numbers of samples in order to identify positive samples. A common technique involves the use of fluorescent or fluorescently labelled reporter molecules which selectively bind only to those sample molecules which have a desired characteristic (eg, DNA sequence, antigen binding site, presence of certain ionic species). A collection of samples is illuminated with light of a specific excitation wavelength; the reporter molecules absorb this light and re-emit light of a second, usually longer, emission wavelength. In this way positive samples can be detected. Common fluorophores attached to reporter molecules include FITC, rhodamine, Cy-5 and Texas Red. Each fluorophore has a specific excitation and emission wavelength. Related techniques to which the present invention may be applied include, but are not limited to, enhanced chemiluminescence detection, ELISA, fluorescence in-situ PCR, and in vivo GFP techniques. The present invention could also be used in the photoactivation of suitable functional surfaces to selectively immobilise chemical or biological species such as DNA, ligands, antibodies and antigens etc.

Multiple samples are often screened in standard-format 96-well plates; or increasingly, compatible format high density plates, where each of the 96 'wells' is actually an array of multiple wells.

In order to screen such large numbers of samples, each sample must be illuminated by the excitation radiation. Typically the entire plate is illuminated simultaneously, and the emission radiation detected by,

for example, a photographic plate. However, the materials used for manufacture of microtitre plates typically have some degree of autofluorescence, that is, they will fluoresce to some extent. This autofluorescence will often be sufficient to obscure a weak or faint signal, so leading to false positive and negative results.

An alternative method is for a single beam of excitation radiation to illuminate each well individually in sequence; however, this may be relatively slow, and requires the excitation radiation beam to be moved precisely, exposing the system to the risk of mechanical failure.

It is among the objects of embodiments of the present invention to alleviate or obviate these and other disadvantages of existing assay systems.

This is achieved by diffracting a single excitation beam of radiation into multiple excitation beams, the spatial location of which correspond with the location of samples to be assayed.

According to a first aspect of the present invention there is provided an apparatus for assaying samples, the apparatus comprising excitation means for emitting radiation of a first excitation wavelength, diffracting means for diffracting the excitation radiation in a radiation pattern, sample presentation means for presenting samples to be assayed, wherein the excitation radiation pattern coincides in location with the sample containing means, and detection means for detecting radiation of at least one emitted wavelength emitted by the samples, whereby, in use, the excitation radiation pattern creates emitted radiation of at least a second wavelength from the samples which is detected by said detection means.

The apparatus of the present invention may conveniently be used in fluorescent biochemical assays, wherein positive samples are labelled by means of a

fluorophore, a chemical group which upon excitation by radiation of a particular wavelength emits radiation at the second, usually longer, wavelength. The apparatus of the present invention may also be used in assays wherein samples are labelled within two or more fluorophores, such that the emitted radiation comprises a plurality of wavelengths. These wavelengths may be detected simultaneously. Where a plurality of wavelengths are to be detected, conveniently the apparatus further comprises an emission filter means for selectively admitting emitted radiation of one of said plurality of emitted wavelengths.

Preferably, the apparatus further comprises a filter to reduce the amount of excitation radiation reaching the detection means.

Preferably, the sample presentation means comprises multiple sample-receiving areas.

Preferably the diffracting means is a diffractive optical element comprising a diffraction grating etched on the surface of a radiation-transparent plate. Conveniently the plate is a quartz plate.

Preferably the plate is a frequency dependent substrate, such that applying energy at a given selected frequency to the plate will cause the plate to alter its conformation, and so the conformation of the diffraction grating. This will enable the excitation radiation pattern to be altered with use of a single diffraction means. Conveniently, the frequency-dependent substrate is a piezoelectric material such that application of electrical energy to the substrate will cause the substrate to deform.

Preferably, the diffracting means diffracts a single input radiation beam of a defined wavelength into a defined multiple beam pattern. Most preferably this defined multiple beam pattern corresponds with the pattern of the multiple sample-receiving areas. The person of skill in the art will be aware of techniques

whereby a diffractive optical element of any desired properties may be constructed.

In order to design such a diffractive optical element, the complex wave amplitude description of the desired image is inverse-propagated, for example, by means of an inverse Fourier or Fresnel transformation, from the image plane to the diffractive element.

The pattern thus defined may then be created on the substrate by means of, for example, photolithography and reactive ion etching, embossing techniques, ion-beam-milling, diamond turning or contact printing.

When etching techniques are used, the depth of the etch corresponds to an optical phase change ϕ, where:

$$\phi = \frac{2\pi h(n_s - 1)}{\lambda}$$

where n_s, is the index of the diffractive element, h is the etch depth, and λ is the wavelength of operation. In this case, the diffractive element is binary. If the process is repeated the diffractive element has four phases, and if three levels of lithography are used the diffractive element has eight phases.

Preferably, the apparatus further comprises an additional diffractive optical element which diffracts the multiple beam pattern into a parallel beam pattern. This may be a separate lens, or may conveniently be formed integrally with the diffracting means.

Preferably, the apparatus further includes multiple diffractive optical elements, organised in a spatial arrangement corresponding to the arrangement of the multiple sample-receiving areas for collecting and focussing radiation emitted by each sample. Conveniently, these multiple diffractive optical elements are formed integrally with the sample-containing means. The multiple diffractive optical elements may conveniently be multiple Fresnel lenses.

Conveniently, the radiation detection means may be photo-multiplier tubes, photo-diodes, a linear diode array, or photographic film or plates, but is preferably a CCD array.

While the above-described apparatus is suitable for assays in which the emitted radiation is of a single wavelength, in some applications it may be desirable to assay samples in which multiple fluorophores are used, and so the emitted radiation is of multiple wavelengths, and multiple excitation wavelengths may also be necessary.

According to a second aspect of the present invention there is provided apparatus for assaying samples comprising a first and a second excitation means for emitting radiation at a first and a second wavelength respectively, a first and a second diffracting means for diffracting the respective first and second excitation radiation emissions in respective first and second radiation patterns, sample presentation means for presenting samples to be assayed, wherein the first and second diffracted excitation radiation emissions coincide in location with the sample presentation means, filter means for selectively admitting emitted radiation of one of said first and second emitted wavelengths, and detection means for detecting radiation emitted by the sample.

Conveniently, the filter means comprises a further diffractive optical element for diffracting radiation of the first and second emitted wavelengths to different extents, thereby creating two distinct signal patterns on the detection means, one for each emitted wavelength.

The apparatus of the present invention may be used with many types of samples; typically the presentation means may be 96-well plates, or high-density microtitre plates; however, the apparatus is suitable for use with such samples as liquids, gels, nylon membranes, solid supports, and DNA chips, among others.

According to a further aspect of the present invention there is provided a method of assaying multiple samples simultaneously, the method comprising the steps of:

providing at least one source of excitation radiation of at least a first wavelength;

diffracting the excitation radiation into multiple radiation beams, the spatial pattern of the beams corresponding to a spatial arrangement of multiple samples;

exciting the samples by the excitation radiation, and detecting radiation of at least a first emitted wavelength emitted by the samples.

Preferably, the spatial arrangement of multiple samples is a two-dimensional array of samples.

Preferably, two sources of excitation radiation are provided, one source providing excitation radiation at a first wavelength and the other source providing excitation radiation at a second wavelength. Preferably also, radiation of said first and said second emitted wavelengths is detected.

According to a further aspect of the present invention there is provided a sample holder for use with apparatus for assaying samples having excitation means for emitting excitation radiation and detection means for detecting radiation emitted in use by samples, the sample holder having multiple sample-receiving areas and multiple optical elements arranged in locations corresponding to the sample-receiving areas, each of which, in use, collects and focuses radiation emitted by each sample for detection by said detection means.

Preferably the multiple sample-receiving areas are arranged in a two-dimensional array.

The sample holder may further include an additional diffractive optical element for diffracting excitation radiation, in use, to form a radiation pattern corresponding to the multiple sample-receiving areas.

According to a still further aspect of the present invention there is provided a diffractive element holder for use with an apparatus for assaying samples including excitation means for emitting excitation radiation, sample presentation means, and detection means for detecting radiation emitted in use by samples; wherein the diffractive element holder comprises a plurality of spaced substantially coplanar diffractive optical elements, each respective diffractive optical element being adapted to diffract radiation of a particular wavelength into a respective radiation pattern, the location of each diffractive optical element being adjustable with respect to said sample presentation means so as to enable each element to be used independently of the other elements to create its respective radiation pattern.

Preferably the respective radiation patterns are substantially identical. Conveniently, the respective patterns have substantially identical footprints.

In one embodiment of the present invention, the diffractive optical elements are formed on separate substrates; in an alternative embodiment, the multiple diffractive optical elements are formed on a single substrate. Conveniently, this is achieved by etching a diffraction grating on a substrate such that the depth and arrangement of the etches varies across the length of the diffraction grating.

According to yet another aspect of the present invention, there is provided a method of manufacturing a substrate bearing an array of bound molecules, the method comprising the steps of:

providing at least one source of excitation radiation;

diffracting the excitation radiation into multiple radiation beams, the spatial pattern of the beams corresponding to a desired spatial arrangement of bound molecules;

exciting a substrate bearing unbound molecules with the excitation radiation, so as to activate a photochemical reaction between the unbound molecules and the substrate and to bind the molecules to the substrate on those parts of the substrate excited by the excitation radiation; and

removing any remaining unbound molecules from the substrate.

These and other aspects of the present invention will now be described by way of example only and with reference to the accompanying figures, in which:

Figure 1 shows a schematic diagram of a system for assaying samples according to a first embodiment of the present invention;

Figure 2 shows an apparatus for assaying samples according to the system outlined in Figure 1;

Figures 3a and 3b show respective enlarged sectional views of two embodiments of a sample holder suitable for use with the apparatus of Figure 2;

Figure 4 shows a diagram of an apparatus for assaying samples according to a second embodiment of the present invention;

Figures 5a and 5b show two focussing structures for providing parallel excitation beams for use with the present invention;

Figure 6 shows a diffractive element holder of multiple diffraction gratings for use with the apparatus of Figure 2 or Figure 4 in accordance with a further embodiment of the present invention;

Figure 7 shows an alternative diffractive element holder;

Figure 8 shows a further embodiment of an apparatus for assaying samples in accordance with an aspect of the present invention; and

Figure 9 is a sketch showing the use of an embodiment of the present invention in a surface plasmon resonance assay.

Referring first to Figure 1, this shows the general layout of apparatus 10 for assaying samples according to a first embodiment of the present invention. The apparatus 10 comprises an excitation means 12, such as a laser, for emitting excitation radiation of a specific wavelength. A beam of excitation radiation 14 is emitted and directed toward a radiation-transparent plate 16, with a diffraction grating 18 etched on the surface of the plate 16. The excitation radiation 14 is diffracted by diffraction grating 18 into a number of separate excitation beams 20. The diffraction grating 18 is constructed in such a manner that the pattern of the radiation beams 20 corresponds to the pattern of sample wells 22 on a sample plate 24 located at a specific distance from the diffraction grating 18. In use, each sample well 22 contains a sample to be assayed, the positive samples of which include a fluorophore which is excited by the radiation beams 20, and emits emission radiation 26. This radiation 26 is detected by a detector 28, which is connected to an image processor 30, whereby the results of the assay may be studied and analysed.

Figure 2 shows a more detailed diagram of an apparatus as described above. The apparatus 40 includes an excitation radiation source 42 connected to a power supply 44. In this embodiment, the radiation source 42 is a HeNe laser, emitting radiation 46 at a wavelength of 632nm with a beam diameter of 0.5mm-5mm. A diffraction grating is etched on a quartz plate 48, designed specifically for use with 632nm light, at a predetermined distance from both radiation source 42 and sample holder 50. It is a straightforward matter for those of skill in the art to design diffraction gratings having the desired characteristics, by means of tools such as are known in the art, as described above.

The quartz plate 48 diffracts the radiation 46 into a pattern 49 corresponding to the pattern of sample wells

51 on a standard 96-well plate such as sample holder 50.

Each excitation beam 49 excites a sample 52 in well 51, which thereby emits emission radiation 54. In this example, Cy-5 is used as a fluorophore, which absorbs light at 632nm, and emits light at 640nm to 800nm with a pH and local environment dependent peak about 670nm. The emission radiation 54 passes through the lower surface of the sample holder 50, which in this embodiment incorporates a number of Fresnel lenses 56 to focus the light from each sample. The focussed light 58 is directed through an emission filter 59, which allows only emitted light to continue toward a CCD array 60, which detects the positive signals and which is connected to a personal computer 62, which processes the signals.

In an alternative embodiment of the present invention, the emission filter 59 may be omitted, and the apparatus used with non-fluorescently-labelled samples 52. The CCD array 60 will then detect excitation radiation which is not absorbed by the samples 52. Such an embodiment is suitable for use in absorbence assays, such as ELISAs. A further variant replaces emission filter 59 with an excitation filter which will only pass excitation radiation, thereby reducing the effects of any unwanted sample fluorescence.

Referring now to Figure 3a, this shows a section of the 96-well plate 48, including a number of wells 51, formed with Fresnel lenses 56 on the lower surface. The diagram shows how light 54 emitted by a sample 52 radiates in all directions from the sample, but is generally focussed to a point 80 by the lens 56, in combination with mirrored walls 53 of the wells 51.

Figure 4 shows an apparatus for assaying samples according to another embodiment of the present invention. The apparatus 100 is suitable for detection of two fluorophores simultaneously. The apparatus 100 includes two light sources 102,104, producing light 106,108 at different wavelengths. Two diffraction gratings 110 and

112 are provided, each designed for use with a specific wavelength of light. The diffraction gratings are designed so as to diffract light from each light source into the same pattern on the same sample holder 114. In this embodiment, each sample contains two fluorophores which are excited at different, first wavelengths, and emit at different, second wavelengths. The mixed emitted light 116 passes through and is focussed by Fresnel lenses 118, and then to a further diffraction grating 120 which serves as a filter. The grating 120 diffracts light of different wavelengths by different amounts, so producing two beams of emitted light 122, 124 from each well, and generating multiple non-overlapping signals from each sample on the CCD array 126. In this manner multiple fluorophores may be detected simultaneously.

Turning now to Figure 5a, this shows a focussing structure for obtaining parallel excitation beams. For many applications it may be desirable for the excitation beams to be made parallel before contacting the sample holders. Figure 5a shows schematically a system 130, whereby a diffraction grating 132 produces multiple divergent beams 134. Use of a convex lens 136 collimates the beams 134, such that the beams may be used to excite samples at any distance from the diffraction grating 132. The lens 136 may be used with the apparatus of Figure 2, and is located between the sample holder 50 and the diffractive element 132,48.

Figure 6 shows a diffractive element holder for receiving several different gratings for use with the apparatus of the embodiments described above. It is often of use to be able to excite samples with different wavelengths of light, in order to detect different fluorophores. As each wavelength of light requires a specifically-made diffraction grating, the holder 150 containing a number of different gratings 152 is of great benefit. The holder may be rotated in use to select the appropriate grating by bringing the desired grating 152

into alignment with the excitation beam source and the sample holder.

Figure 7 shows an alternative embodiment of a diffractive element holder 160 located over a microtitre plate 161. In this example, the element holder 160 comprises a single substrate 162, on which is etched a diffractive grating pattern 164 which varies over the length of substrate 162. Thus, a user may move the holder 160 in the direction of arrows A to make use of the varying properties of the diffraction grating and, consequently, change the radiation pattern reaching plate 161.

Figure 8 shows an embodiment of the present invention in the form of an apparatus for assaying epifluorescence of a sample. The apparatus 180 is similar to those described above, comprising a light source 182, an excitation filter 184, a collimating lens arrangement 186, a diffraction grating 188, a sample holder 190, and a CCD array 192.

The epifluorescence apparatus differs from those described previously in that a dichroic mirror 194 is provided between the diffraction grating 188 and sample holder 190, which reflects excitation radiation toward the sample holder 190, while emission radiation from the sample is allowed to pass through the dichroic mirror 194 where it is directed by a further series of mirror 196, lens 198, and emission filter 200 towards the CCD array 192.

An alternative arrangement may be made for measuring fluorescence polarisation of a sample, by the addition of a polariser 202 in the excitation path, and an analyser 204 (essentially another polariser) in the emission path. The polariser 202 and analyser 204 are marked on figure 8 in dotted outline. Upon excitation with a polarised beam, fluorescence emission is partially polarised. The degree of polarisation is related to rotational correlation time of an analyte. Therefore, the degree of

binding between two molecules in a sample may be measured; the present invention allows multiple readings to be made simultaneously.

A yet further application of the present invention is illustrated in figure 9, which shows the measurement of surface plasmon resonance (SPR) of a sample. Multiple parallel excitation beams 210 are showing impinging on a test sample at an angle θ. The test sample comprises a translucent dielectric 212, such as glass, with a surface layer 214 of metal, such as gold, of a thickness of a few nanometers. If the angle θ is selected correctly, (dependent on the metal used and the desired depth of penetration) the excitation radiation generates an evanescent electric field 213 on the surface layer 214 of gold which propagates along the surface layer 214 as shown. This field 213 is sufficient to excite samples 216 bound to the gold layer 214, which then generates an emission radiation 218, which is detected by a CCD detector 220. Multiple samples may be detected simultaneously, using the multiple parallel beams generated by the embodiments of the present invention. An variation of this embodiment of the invention makes use of the total internal reflective properties of a transparent dielectric to excite samples bound directly to the surface of such a dielectric 212, rather than to a gold surface layer 214 because the electric field generated by the impinging excitation beams is sufficient to penetrate the dielectric surface and excite the bound samples.

It will be apparent to those of skill in the art that various modifications and improvements may be made to the apparatus and method described herein without departing from the scope of the invention.

For example, a sample holder for use with the present invention may include integral Fresnel lenses to focus the emitted radiation, as described above. Alternatively, a focussing mechanism may be provided

separately of the sample holder; or, for some applications, a focussing mechanism may not be necessary and is not provided. Each Fresnel lens may also be provided with an individual integral detection means.

Figure 3b shows a section of an alternative sample holder 90; in this case a support for electrophoretic gels 92. The gel 92 contains DNA or RNA fragments 94, which are illuminated by excitation radiation 96. In this embodiment, the lenses 98 are formed separately from the sample holder 90.

Concerning the embodiment of Figure 4, for detecting multiple fluorophores, the diffraction grating 120 may be replaced by a pair of interchangeable filters which each admit light of only one wavelength. In this manner, the signal from each fluorophore may be detected individually.

For those applications in which collimated excitation beams are necessary, an alternative collimating structure 140 is shown in Figure 5b. In this embodiment, the plate 142 on which the diffraction grating is etched is itself lenticular, thereby automatically collimating the beams 144 as they leave the diffraction grating.

The embodiments thus far described have one or two diffractive optical elements for splitting excitation radiation into a defined, fixed multiple beam pattern. In an alternative embodiment the diffractive optical element includes a piezoelectric plate as a substrate. Varying the frequency of electricity applied to the plate causes the plate to alter conformation which, in turn, alters the multiple beam pattern produced by the plate. This enables a single diffractive optical element to be used with a number of alternate arrangements of samples, so providing greater flexibility to the system.

The diffractive element holder described above may be produced for use with the system of Figure 4, wherein multiple gratings may be used simultaneously with

separate light sources. Selectable pairs of gratings may of course be provided. Furthermore, the holder need not be rotatable: a linear arrangement of gratings may be provided, or indeed a column of gratings, one of which may be aligned with the light source and the sample holder.

While the present invention has been described for use in an assay system, it will be understood that the invention is not limited to this use. For example, the apparatus and method have an application in photochemistry, where light or other electromagnetic radiation of a specific wavelength may be used to cause a chemical reaction in an array of defined locations.

For instance an array of molecules may be built up on a substrate by coating the substrate with a solution of photoactivatable molecules, illuminating the substrate in a predetermined pattern by means of a diffraction grating and laser, substantially as described above, such that those areas which are illuminated are activated to bind the molecule to the substrate, and washing off the unbound molecules. This process may be repeated several times, with different diffraction gratings if desired or by rotating the diffraction grating or substrate between exposures, in order to build up a complex array of bound molecules. Such a method is of use in assembling DNA "chips", or combinatorial chemistry libraries, and the like, by sequentially binding layers of nucleotides or amino acids for example.

It should be understood that for such synthetic uses of the present invention (unlike the analytical uses described above), it is unnecessary to provide a detector arrangement in these embodiments.

Although the majority of excitation radiation passing through the diffraction grating will be diffracted, some will not be diffracted, and will pass directly to the sample. Such "zeroth order" radiation may be used in any of the embodiments of the present

invention to calibrate the amount of radiation reaching the sample, or to provide an alignment marker for aligning sample holders with the diffracted excitation radiation.

A further use of the present invention is with turbidity or nephrometry assays.

For all aspects of the invention, the beam diameter and diffracted beam pattern may be varied by adjusting the ratio of focal lengths of the objective lenses as shown in, for example, figure 8, or by varying the position of the diffraction grating.

It will be apparent to those of skill in the art that the present invention provides an assay system whereby multiple samples may be assayed simultaneously and rapidly, with a high signal-to-noise ratio, high sensitivity, high speed, and reliably, as there are no moving parts to the system.

CLAIMS

1. An apparatus for assaying samples, the apparatus comprising excitation means for emitting radiation of a first excitation wavelength, diffracting means for diffracting the excitation radiation in a radiation pattern, sample presentation means for presenting samples to be assayed, wherein the excitation radiation pattern coincides in location with the sample presentation means, and detection means for detecting radiation of at least one emitted wavelength emitted by the samples, whereby, in use, the excitation radiation pattern creates emitted radiation of at least a second wavelength from the samples which is detected by said detection means.

2. The apparatus of claim 1, further comprising an emission filter means for selectively admitting emitted radiation of one of said plurality of emitted wavelengths.

3. The apparatus of claim 1 or claim 2 further comprising a filter to reduce the amount of excitation radiation reaching the detection means.

4. The apparatus of any preceding claim, wherein the sample presentation means comprises multiple sample-receiving areas.

5. The apparatus of any preceding claim, wherein the diffracting means is a diffractive optical element comprising a diffraction grating etched on the surface of a radiation-transparent plate.

6. The apparatus of claim 5 wherein the plate is a quartz plate.

7. The apparatus of claim 5 or claim 6, wherein the

plate is a frequency dependent substrate, such that applying energy at a given selected frequency to the plate will cause the plate to alter its conformation, and so the conformation of the diffraction grating.

8. The apparatus of claim 7, wherein the frequency-dependent substrate is a piezoelectric material such that application of electrical energy to the substrate will cause the substrate to deform.

9. The apparatus of any preceding claim, wherein the diffracting means diffracts a single input radiation beam of a defined wavelength into a defined multiple beam pattern.

10. The apparatus of claim 9, wherein said defined multiple beam pattern corresponds with the pattern of the multiple sample-receiving areas.

11. The apparatus of claim 9 or claim 10 further comprising an additional diffractive optical element which diffracts the multiple beam pattern into a parallel beam pattern.

12. The apparatus of claim 11, wherein the additional diffractive optical element is a separate lens.

13. The apparatus of claim 11, wherein the additional diffractive optical element is formed integrally with the diffracting means.

14. The apparatus of any preceding claim, further comprising multiple diffractive optical elements, organised in a spatial arrangement corresponding to the arrangement of the multiple sample-receiving areas for collecting and focussing radiation emitted by each sample.

15. The apparatus of claim 14, wherein said multiple diffractive optical elements are formed integrally with the sample-containing means.

16. The apparatus of claim 15, wherein the multiple diffractive optical elements are multiple Fresnel lenses.

17. The apparatus of any preceding claim wherein the radiation detection means comprises a CCD array.

18. The apparatus of any preceding claim, further comprising polarising filters in both the excitation and emission radiation paths.

19. The apparatus of any preceding claim, further comprising a dichroic surface disposed so as to direct excitation radiation to the sample presentation means, and to direct emitted radiation to the detection means.

20. An apparatus for assaying samples comprising a first and a second excitation means for emitting radiation at a first and a second wavelength respectively, a first and a second diffracting means for diffracting the respective first and second excitation radiation emissions in respective first and second radiation patterns, sample presentation means for presenting samples to be assayed, wherein the first and second diffracted excitation radiation emissions coincide in location with the sample presentation means, filter means for selectively admitting emitted radiation of one of said first and second emitted wavelengths, and detection means for detecting radiation emitted by the sample.

21. The apparatus of claim 18, wherein the filter means comprises a further diffractive optical element for diffracting radiation of the first and second emitted wavelengths to different extents, thereby creating two

distinct signal patterns on the detection means, one for each emitted wavelength.

22. A method of assaying multiple samples simultaneously, the method comprising the steps of:

providing at least one source of excitation radiation of at least a first wavelength;

diffracting the excitation radiation into multiple radiation beams, the spatial pattern of the beams corresponding to a spatial arrangement of multiple samples;

exciting the samples by the excitation radiation, and detecting radiation of at least a first emitted wavelength emitted by the samples.

23. The method of claim 22 wherein the spatial arrangement of multiple samples is a two-dimensional array of samples.

24. The method of claim 22 or claim 23, wherein two sources of excitation radiation are provided, one source providing excitation radiation at a first wavelength and the other source providing excitation radiation at a second wavelength.

25. The method of claim 24 wherein radiation of said first and said second emitted wavelengths is detected.

26. The method of claims 22 to 25 wherein the assay is a surface plasmon resonance assay.

27. The method of claims 22 to 26 further comprising the step of providing a dichroic surface in the path of both the excitation radiation and the emission radiation, so as to direct the excitation radiation to the samples, and to direct the emission radiation to a detector means.

28. A sample holder for use with apparatus for assaying samples having excitation means for emitting excitation radiation and detection means for detecting radiation emitted in use by samples, the sample holder having multiple sample-receiving areas and multiple optical elements arranged in locations corresponding to the sample-receiving areas, each of which, in use, collects and focuses radiation emitted by each sample for detection by said detection means.

29. The sample holder of claim 28, wherein the multiple sample-receiving areas are arranged in a two-dimensional array.

30. The sample holder of claim 28 or claim 29 further comprising an additional diffractive optical element for diffracting excitation radiation, in use, to form a radiation pattern corresponding to the multiple sample-receiving areas.

31. A diffractive element holder for use with an apparatus for assaying samples including excitation means for emitting excitation radiation, sample presentation means, and detection means for detecting radiation emitted in use by samples; wherein the diffractive element holder comprises a plurality of spaced substantially coplanar diffractive optical elements, each respective diffractive optical element being adapted to diffract radiation of a particular wavelength into a respective radiation pattern, the location of each diffractive optical element being adjustable with respect to said sample presentation means so as to enable each element to be used independently of the other elements to create its respective radiation pattern.

32. The diffractive element holder of claim 31, wherein the respective radiation patterns are substantially

identical.

33. The diffractive element holder of claim 31 or claim 32 wherein the respective patterns have substantially identical footprints.

34. The diffractive element holder of claims 31 to 33 wherein the multiple diffractive optical elements are formed on a single substrate.

35. The diffractive element holder of claim 34, wherein a diffraction grating is etched on a substrate such that the depth and arrangement of the etches varies across the length of the diffraction grating.

36. A method of manufacturing a substrate bearing an array of bound molecules, the method comprising the steps of:

providing at least one source of excitation radiation;

diffracting the excitation radiation into multiple radiation beams, the spatial pattern of the beams corresponding to a desired spatial arrangement of bound molecules;

exciting a substrate bearing unbound molecules with the excitation radiation, so as to activate a photochemical reaction between the unbound molecules and the substrate to bind the molecules to the substrate on those parts of the substrate excited by the excitation radiation; and

removing any remaining unbound molecules from the substrate.

37. The method of claim 36 wherein the molecules are nucleic acid molecules.

38. The method of claims 36 or 37 wherein the steps are

repeated several times with distinct molecular species so as to generate an array of distinct bound molecular species.

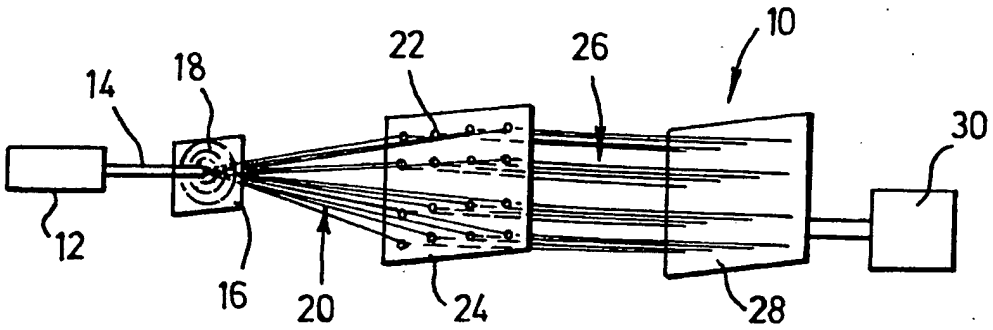
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Fig.1

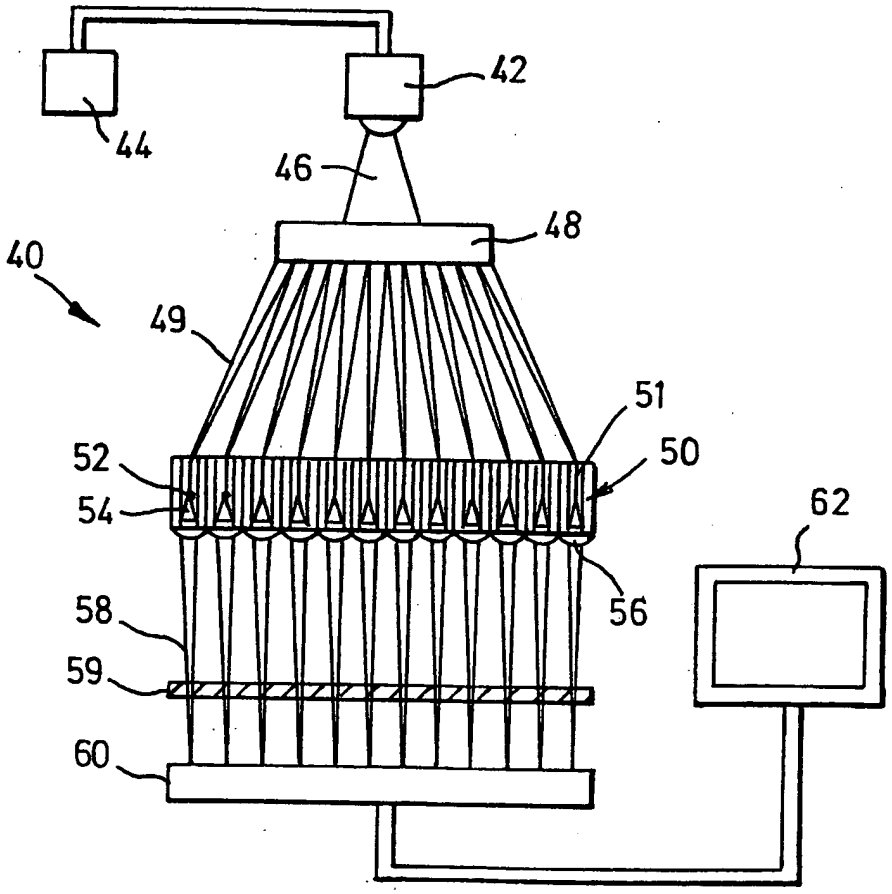
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Fig.2

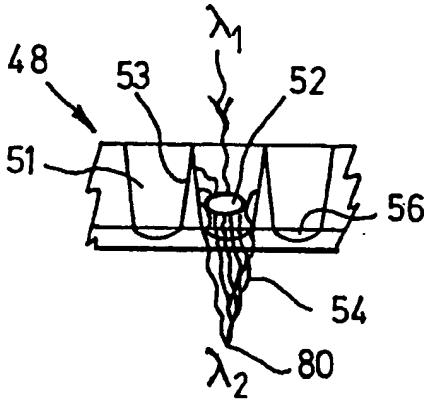

Fig.3a

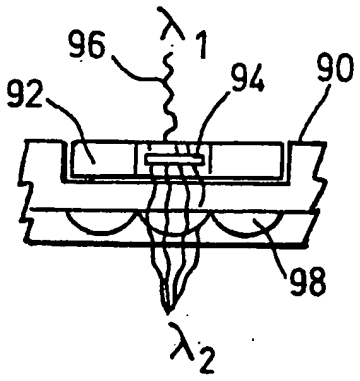

Fig.3b

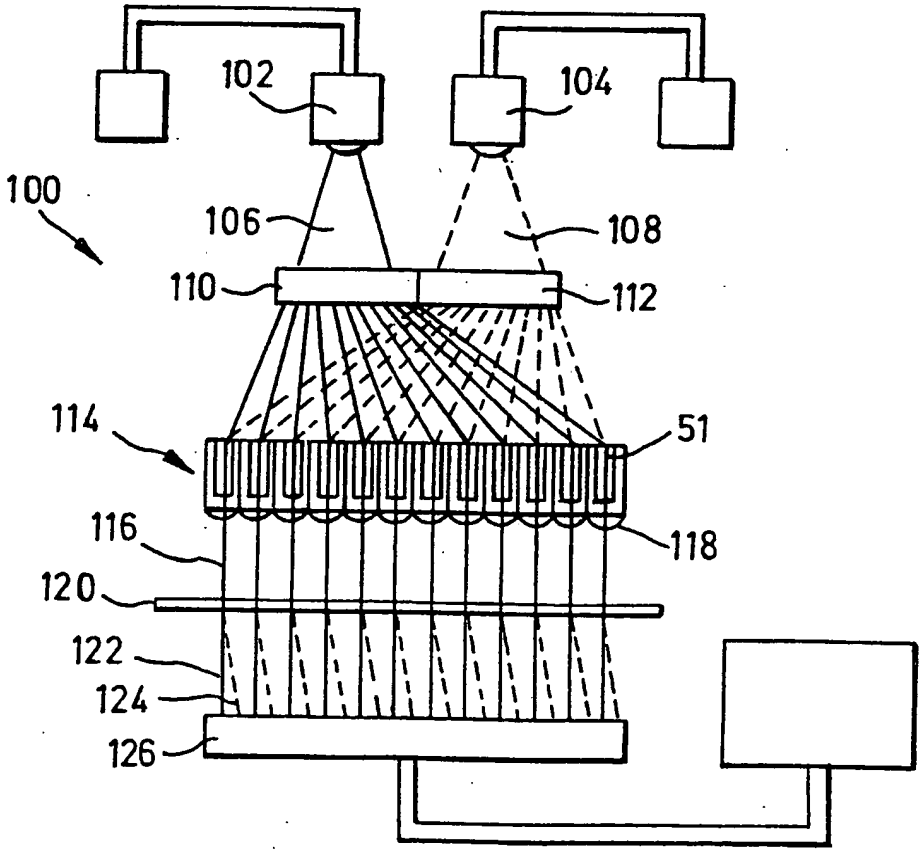

Fig.4

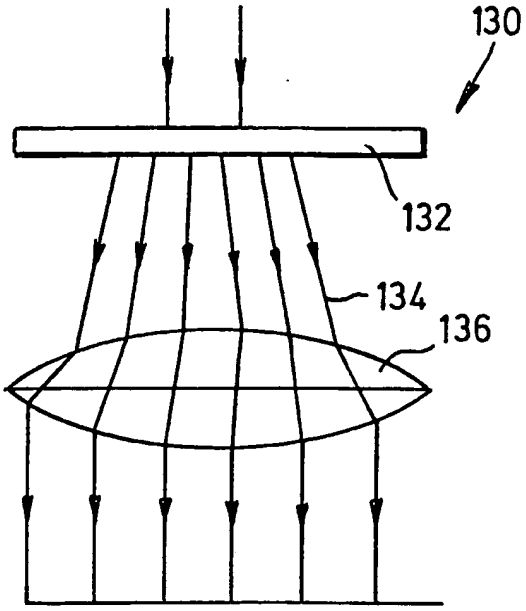

Fig.5a

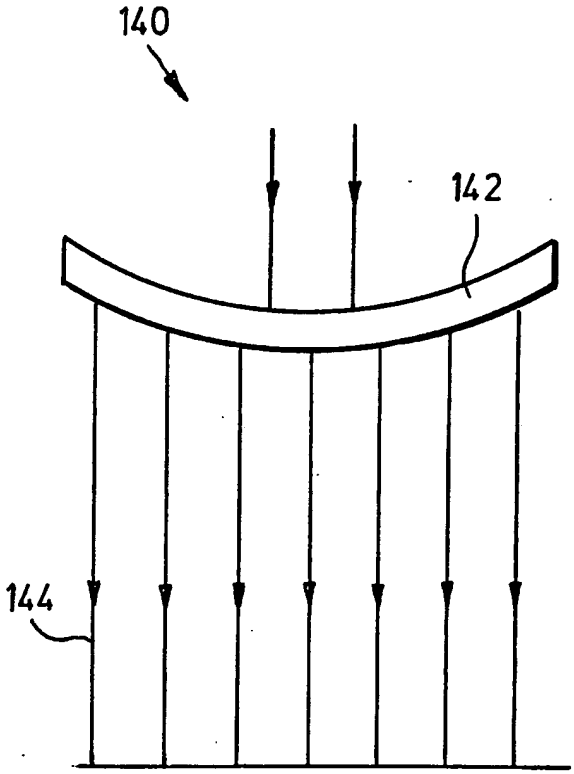

Fig.5b

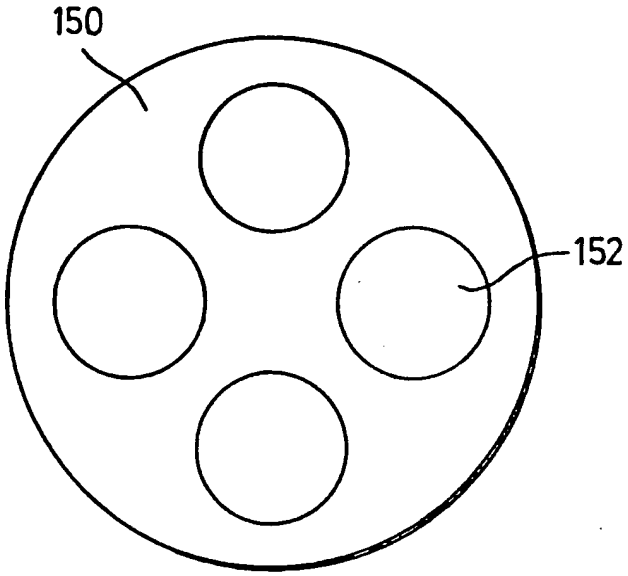

Fig.6

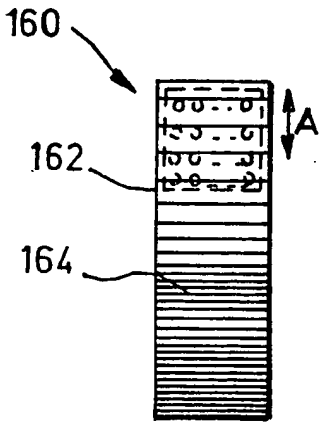

Fig.7

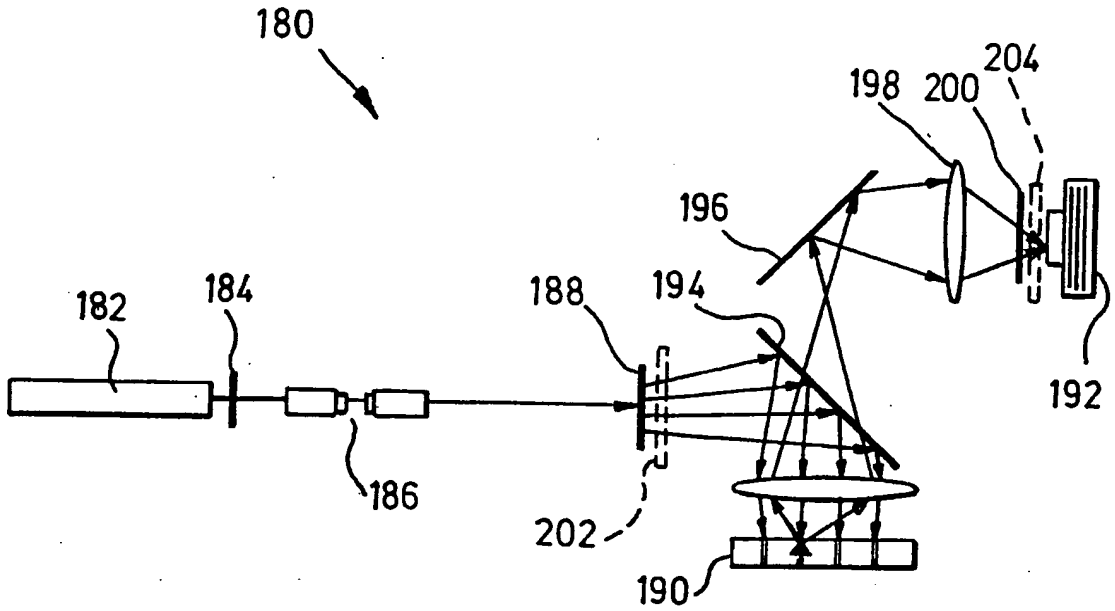
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Fig.8

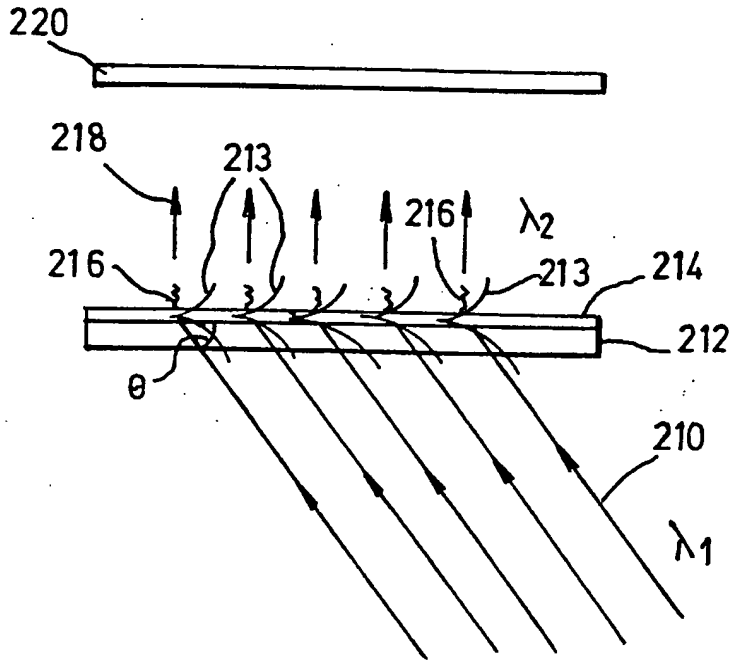
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Fig.9